SURVEY FOR INTERGENERIC POLLEN TUBE GROWTH IN INTERGENERIC POLLINATIONS UTILIZING THE *IAP* ALLELE IN *SORGHUM BICOLOR*

A Thesis

by

MATTHEW SCOTT BARTEK

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2010

Major Subject: Plant Breeding

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Approved by:

Chair of Committee, William L Rooney
Committee Members, Byron L. Burson
Stephan L. Hatch
Head of Department, David D. Baltensperger

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ABSTRACT

Survey for Intergeneric Pollen Tube Growth in Intergeneric Pollinations Utilizing the *iap* Allele in *Sorghum bicolor*. (December 2010)

Matthew Scott Bartek, B.S., Texas A&M University

Chair of Advisory Committee: Dr. William Lloyd Rooney

Hybridization within *Sorghum bicolor* (L.) Moench has been the primary means of creating genetic diversity needed for sorghum crop improvement. While significant variation exists within *S. bicolor*, there are several traits that can be improved and potential opportunities to improve *S. bicolor* if secondary and tertiary germplasm pools could be accessed. Recently, the discovery of the *iap* (Inhibition of Alien Pollen) mutant and its introgression into more breeding-amenable genetic background has facilitated the development of *S. bicolor* germplasm with genetic diversity not previously seen within *Sorghum.* The key to producing this variation is the homozygous recessive mutant gene *iap* which removes an important reproductive isolation barrier to hybridization. Development of a *S. bicolor* accession (Tx3361) containing the mutant allele *iap* and *ms3* has allowed introgression of genomic regions from divergent sorghum species into *S. bicolor*. Given the success with divergent sorghum species, there is a real interest in assessing the potential of this mutant to facilitate intergeneric hybridization. The objective of this study was to determine the range and effectiveness of the iap mutant to allow pollen tubes of Poaceae species outside of the genus *Sorghum* to grow into *S. bicolor* pistils. Accessions from the

genera *Zea*, *Miscanthus*, *Pennisetum*, and *Sorghastrum* were used as pollen donors onto Tx3361 and fluorescent microscopy was used to determine the distance through the pistils that foreign pollen tubes grew. Results indicate high levels of pollen tube growth into the ovaries of *S. bicolor* pistils for two accessions of *Pennisetum ciliare* (L.) Link and four accessions of *Zea mays* L. Pollen tubes of other accessions tested did grow to the ovary but in very small numbers. While the recovery of embryos was not attempted in this study, the results indicate that there is potential for hybridization, but the specific pollinator within a species is critical in this attempt.

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INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the most widely utilized crops throughout the world; its grain is used as human food and animal feed while the forage is valued as animal fodder. Sorghum breeding over the last century has led to improvements in grain quality and yield. Between 1920 and 1998, sorghum grain yield increased from 224.1 $kgha^{-1}$ to just over 678.5 $kgha^{-1}$ in the United States (Smith, 2000).

Traditionally, genetic improvement of any important crops is achieved by maximizing the available genetic variation within a species (Sharma, 1995). In the case of Sorghum, the primary genetic pool has been the species *S. bicolor*. Breeders have used a wide range of diversity present in this species to make most all of the genetic enhancements that have been accomplished. These efforts have led to the development of sorghums specifically for grain, forage and even industrial purposes. In addition, the genetic systems required for hybrid sorghum production were found within the species.

Having a diverse germplasm pool is a valuable resource, but there are always additional traits that would be desirable but are not available in the primary gene pool. The ability to access traits present in secondary and tertiary gene pools is extremely valuable. Interspecific hybridization is used in crops [such as wheat (*Triticum aestivum* L.), corn (*Zea mays* L.), and sorghum] to move genes or traits that either enhance the crop's potential or protect the intrinsic value of the crop. Examples of wide hybridization within the grass family include; *Saccharum* x *Sorghum* (de Wet et al., 1976), *Triticum* x *Agropyron* (Goodman et al., 1987) and *Zea* x *Tripsacum* (Stalker et al., 1977) in which trait

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This thesis follows the style of Crop Science.

introgression from one genus to another was successful (Dhaliwal and King 1978).

Interspecific hybridization of *S. bicolor* with divergent sorghum species has recently produced diversity not previously seen within *Sorghum* species (Price et al., 2006). Hacker et al. (1992) stated that species belonging to gene pools outside of the *S. bicolor* primary gene pool contain many desirable traits that have not been accessible for sorghum improvement. Increased diversity has potential to improve drought and pest tolerance as well as other desirable agronomic traits. Resulting studies have revealed possible intergeneric hybridization of sorghum with other species within the grass family (Sharma, 1995).

Reproductive barriers often exist between divergent relatives and crop species that make interspecific hybridization difficult to achieve (Hodnett et al., 2005). The presence of such mechanisms is not unexpected; plant species must develop mechanisms to protect the viability of the progeny that they produce. However, it is essential to overcome these reproductive barriers to recover interspecific hybrids between *S. bicolor* and its wild relatives. One way to minimize these barriers is to look for genotypes that lack one or more of these barriers (Price et al., 2006).

Price et al. (2006) screened germplasm and identified a specific line that lacks this barrier trait and facilitates much higher rates of hybridization. This trait was designated *iap* (Inhibition of Alien Pollen). Kuhlman et al. (2010) described the development of Tx3361 sorghum germplasm that possesses the trait in an improved agronomic background. To date, Tx3361 has been used to produce intergeneric hybrids of sorghum/sugarcane (Hodnett et al., 2010). There is a real need to fully determine the effect of *iap* in Tx3361 facilitated hybrids beyond the sorghum and saccharum genuses (Price et al., 2006). The objectives of this study are:

1. To screen accessions from different genera within Poaceae to determine the potential for alien pollen tube growth of these genera in Tx3361.
2. Compare foreign pollen tube growth in pistils of Tx3361 and ATx623 to determine the relative effectiveness of *iap* in allowing the foreign pollen to germinate and grow on sorghum stigmas.

REVIEW OF LITERATURE

Taxonomy of Poaceae

The family Poaceae, often referred to as the "grass family", is one of the most economically important and widespread plant families in the world. Members of this family inhabit nearly one-third of the earth's arable dry land (Mathews et al., 2000). Arguably Panicoideae is the most important subfamilies with regards to human sustenance. Aside from being consumed by most of the human population on a daily basis (Mathews et al., 2000), Panicoideae species are also spread throughout most of the world. They are found on all human inhabited continents and they are prevalent within the warm, temperate regions of the world. Corn, sorghum, sugar cane (*Saccharum officinarum* L.) and pearl millet [*Pennisetum glaucum* (L.) R. Br.] are just a few of the species that make this sub-family so vital to our existence (Giussani et al., 2001). Taxonomic classifications within Poaceae were constructed using morphological characteristics of grass structures (Mathews et al., 2000); more modern approaches such as anatomy and cytology further defined these families and how they are related. Most recently, resolution among genealogical and evolutionary relationships within the family have been identified using DNA sequencing. By utilizing the phylogenetic analysis of partial phytochrome B (PHYB) nuclear DNA sequences, Mathews et al. (2000) presents evolutionary relationships of genera within Poaceae. The use of DNA sequence analysis allows consistent phylogenetic relationships to be derived and represent a taxonomic system that reflects evolutionary patterns within Poaceae. The eight subfamilies derived from Mathews et al. (2000) study are; Anomochlooideae, Pharoideae, Bambusoideae, Pooideae, Centothecoideae, Arundinoideae, Chloridoideae and Panicoideae. The subfamily Panicoideae is divided into three tribes; Andropogoneae, Arundinelle, and Paniceae with

Sorghum, *Zea* and *Miscanthus* belonging to the tribe Andropogoneae and *Pennisetum* belonging to the tribe Paniceae.

In the subfamily Panicoideae, sexually reproducing species vary as to their methods of pollination. There are species within the subfamily that propagate either vegetatively or via apomixis; in these species, sexual reproduction is rare. In many others, sexual propagation via self pollination and/or cross pollination is the primary means of reproduction.

Mechanisms of Sexual Incompatibility

Sexual reproduction in plants relies on complex interactions between the pistil and pollen tubes (Lord and Russell, 2002). The pistil is composed of female reproductive organs; the stigma, style, and ovary. Each pistil contains two stylodia with the upper part of each stylodia constituting a stigma. Secondary stigma branches protrude outward from each stigma creating pollen binding sites (Heslop-Harrison, 1982). The style is part of the stylodia but contains no secondary stigma branches. The style connects the stigma with the ovary (Heslop-Harrison, 1982). An unfertilized egg is in an embryo sac encased in an ovule located within the ovary. Pollen tubes grow directionally from the stigma into the ovary (Cheung, 1996). Due to uniformity within the Poaceae family, these findings can be generalized to all genera within the family (Heslop-Harrison, 1982).

Pollination is initiated the moment pollen adheres to the surface of secondary stigma branches (Cheung, 1996). Following adhesion to the stigma, pollen grains are hydrated by imbibing exudates from the surface of secondary stigma branches which initiates germination. Extrusion of the pollen tubes from the pollen grains completes the process of

germination (Heslop-Harrison, 1982). After germination, the pollen tubes penetrate the secondary stigma branches and enter the extra cellular matrix (ECM). Pollen tubes grow within the ECM until they enter the embryo sacs (Cheung, 1996). Each pollen tube follows a path within the pistil which leads from the secondary stigma branches to the central axis of the stigma. After entering the stigma, pollen tubes are guided through the style and eventually into the ovary (Edlund et al., 2004). Upon entering the ovary, pollen tubes grow to its base and enter the embryo sac through the micropyle. The micropyle is a small opening in the ovule controlling orientation of pollen tube entry into the embryo sac. The micropyle also regulates the number of pollen tubes entering the embryo sac. Once within the embryo sac, the pollen tube will penetrate the egg cell and rupture, releasing male germ cells and completing fertilization (Cheung, 1996).

Fertilization cannot occur if the pollen tubes fail to reach embryo sacs due to reproductive barriers (Heslop-Harrison, 1982). Multiple barriers can exist on and within pistils to prevent undesired pollen tube growth and thus eliminating possible fertilization (Cheung, 1996). These barriers begin at the secondary stigma branches where pollen tubes can be denied adhesion and entry into the pistil. The secondary stigma branches utilize strong adhesive interactions to ensure that compatible pollen grains remain on the pistil. The interactions between pollen and stigma may vary between species (Edlund et al., 2004). Growth may also be ceased within the stylodia or the upper ovary wall. Once inside the ovary, pollen tubes must grow to and enter the micropyle for fertilization to occur (Heslop-Harrison, 1982). Pollen tube entry into the micropyle is guided by female gametophytes (Lord and Russell, 2002). The location and strength of pollen rejection varies from species to species. Failure of alien pollen tubes to grow through the stigma and style has been

reported as the primary barrier to interspecific hybridization within *Sorghum* (Hodnett et al., 2005). Rejection has also been observed to be affected by individual genotypes within a population (Heslop-Harrison, 1982).

A second reason that interspecific and intergeneric crosses often fail is embryo abortion after fertilization (Heslop-Harrison, 1982). In many cases, the abortion is due to endosperm breakdown (Price et al., 2006). It is possible to rescue embryos and culture them in vitro, as was done by Price et al. (2006) to recover interspecific hybrids within the *Sorghum* genus. Pre- and post-fertilization barriers vary in strength and location between species but overcoming these barriers can be achieved through scientific progress and application (Sharma, 1995).

Interspecific and Intergeneric Hybridization in Poaceae

Wide hybridization in plant breeding is considered to be a useful tool for creating new species, gene transfer, or induction of haploids, but little is known about the possible range at which wide hybridization is successful (Zenkteler and Nitzsche, 1983).

Harlan et al. (1973) suggested using gene pool classifications to uniformly discuss the genetic compatibility within cereals. Gene pools were designated as primary, secondary and tertiary. Primary pools consist of plant accessions within the same species. Secondary germplasm pools typically consist of species within the same genus, and tertiary pools typically consist of genera within the same family. Members of a primary gene pool are easy to hybridize and produce viable and fertile progeny with Mendelian inheritance and segregation. In a secondary gene pool, hybrids are usually successful, but the gene flow is restricted most typically due to female or male sterility which can be caused by an array of

genetic, cytogenetic and developmental factors. The secondary gene pool is typically different species and these interspecific hybrids have low fertility, high levels of mortality, and poor meiotic chromosome pairing. Harlan et al. (1973) stated "genes can be transferred from the secondary to the primary gene pool but one must struggle with those barriers that separate biological species." Tertiary gene pools are the most challenging to use. Most hybridization attempts between members of the tertiary gene pool are not successful. If successful, a tertiary gene pool cross is almost always sterile. In these situations, introgression is only possible through cytological manipulation. Hybridization attempts of plants within the tertiary gene pool are typically intergeneric crosses (Harlan et al., 1973).

Hybridization of germplasm within a species gene pool has traditionally been used to improve specific traits of the respective crop (Duncan et al., 1991). Interspecific hybridization is a way to introgress genes from wild species into cultivated species of the same genus. Reproductive barriers to hybridization within sorghum involve pre-zygotic as well as post-zygotic mechanisms to prevent fertilization of the ovule by germ cells (Hodnett et al., 2005). Pre-zygotic mechanisms include failure of pollen to germinate, grow through the pistil, and/or enter the embryo and complete fertilization. Post-zygotic mechanisms involve negative genotypic reactions which result in embryo lethality or endosperm abortion which results in embryo death. Hodnett et al. (2005) surmised that the primary barrier to wide hybridization in sorghum is the inability of foreign pollen tubes to grow through the pistils of *S. bicolor*. However, it is essential to overcome these reproductive barriers to recover interspecific hybrids between *S. bicolor* and its wild relatives. The ability of an allele or gene to eliminate or reduce reproductive barriers does occur in other crops.

In wheat, the *Kr*$_1$ and *Kr*$_2$ genes remove many incompatibilities to wide hybridization. Zenkteler and Nitzsche (1983) examined fertilization and embryo development in intergeneric crosses onto rye (*Secale cereal* L.), wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.). It was noticed that fertilization did occur in some instances as indicated by the development of embryos (Zenkteler and Nitzsche, 1983). A previous study by Snape et al. (1979) had also indicated similar success in which substitutions of chromosomes 5A and 5B in certain wheat cultivars allowed hybrid progeny to be recovered when pollinated with barley and rye.

The discovery of a trait, similar to the *Kr*$_1$ and *Kr*$_2$ alleles, was made in sorghum by Laurie and Bennett (1989) although it has not been determined if the two traits are evolutionarily related (Price et al., 2006). Laurie and Bennett (1989) screened accessions of *S. bicolor, S. caffrorum* (Thunb.) P. Beauv., *S. cernuum* (Ard.) Host, *S. dochna* Forrsk, *S. durra* (Forssk.) Stapf, *S. nervosum* Besser ex Schult. & Schult. f., *S. halepense* (L.) Pers., *S. verticilliflorum* (Steud.) Stapf, and *S. versicolor* Andersson (sect. *Parasorghum*) for such variants by pollinating each with *Zea mays* pollen. They identified one *S. nervosum* accession (Nr481) in which the maize pollen tubes grew into a limited number of styles. Analysis of additional Nr481 genotypes revealed the presence of a dominant allele of the gene (*Iap*), that inhibited maize pollen tube growth within the styles of *S. nervosum.* After additional testing of Nr481, a homozygous recessive genotype (*iap,iap*; inhibition of alien pollen) variant was identified that allowed maize pollen tube growth into the styles of this *S. nervosum* accession.

Clearly, the *iap* mutant was of value to interspecific and intergeneric sorghum hybridization. However, the original source selected by Laurie and Bennett (1989) was

agronomically undesirable and did not produce quality progeny or large quantities of seed. Consequently, Kuhlman et al. (2010) utilized original germplasm selected by Laurie and Bennett (1989) to develop Tx3361. Tx3361 is a sorghum germplasm that possesses the *iap* locus in a homozygous recessive condition; it also segregates for genetic male sterility at the Ms_3 locus. The presence of genetic male sterility eliminates the need for emasculation to produce interspecific and intergeneric crosses by simply pollinating genetic male sterile plants. The agronomic properties of Tx3361 are substantially better than those of Nr481; it is similar in maturity and height to existing sorghum inbred lines and has much higher levels of lodging resistance than Nr481 (Kuhlman et al., 2010).

Interspecific Hybridization Involving Sorghum

Until recently, the production of viable interspecific hybrids within the genus *Sorghum* has been rare with only limited reports in the literature, the most notable hybrid being between *S. bicolor* and a weedy wild relative johnsongrass [*Sorghum halepense* (L.) Pers.]. Dweikat (2005) reported interspecific hybridization of these two species which was confirmed by phenotypic and genotypic analyses. Further analysis of the F_1 progeny revealed a high level of male and female fertility. Subsequent self pollination of the F_1 hybrids resulted in seed set more than 90% (Dweikat, 2005). Price et al. (2005) successfully hybridized *S. bicolor* x *S. macrospermum* Garber in which recovery of a single hybrid was achieved through the examination of 1200 *S. bicolor* (ATx623) florets which had been pollinated by *S. macrospermum* for embryo development. Confirmation of hybridization was made through morphological and cytological analysis.

The discovery and use of *iap* has increased the frequency and utilization of interspecific hybridization in sorghum. Price et al. (2006) recovered hybrids when *S. bicolor* (*iap,iap*) was pollinated with *S. angustum* Blake, *S. nitidium* (Vahl) Pers., and *S. macrospermum* pollen. While some required embryo rescue, the level of success using this system was at least two orders of magnitude higher than crosses made by the same species not utilizing the *iap* allele (Price et al., 2006). Kuhlman et al. (2008) also produced *S. bicolor* (*iap,iap*) x *S. macrospermum* hybrids and then successfully made backcrosses to *S. bicolor* in an attempt introgress genes from *S. macrospermum* into cultivated sorghum. Ultimately, introgression was documented, proving that it was possible to introgress significant regions of alien chromosomes into *S. bicolor*, demonstrating the utility and ability of *iap* in wide hybridization of improved versions of *S. bicolor* (Kuhlman et al., 2008).

Intergeneric Hybridization Involving Sorghum

Historically, reports of intergeneric hybridization involving sorghum have been limited. Attempts have been reported with both corn and sugarcane (*Saccharum* spp.), but success has been limited. Dhaliwal and King (1978) experimented with the direct pollination of ovules in reciprocal crosses of *Zea mays*, *Zea Mexicana* (Schrad) H.H. Iltis and *Sorghum bicolor*. They determined there was complete pollen-pistil incompatability in the *sorghum* x *corn* crosses. However, the corn pollen germinated but failed to grow into the sorghum stigmas. It was reported that the barrier to successful sorghum x corn hybridization was the failure of corn pollen tubes to penetrate sorghum pistils (Dhaliwal and King 1978). James (1979) reported successful recovery of 25 maize x sorghum hybrids resulting from 30,000 crosses in which some seed survived to maturity. Hybrid cells contained 20 maize chromosomes and up to 10 sorghum chromosomes. Seed set was recorded for 11 of the

hybrids when backcrossed onto maize. Most backcrossed progeny possessed only 20 maize chromosomes but it was noticed that a single BC_2 plant contained 4 sorghum chromosomes. Backcross progeny morphology was erratic with one reported plant growing to 12cm in height, producing three ears and no tassel.

Two successful productions of intergeneric sorghum hybrids involved reciprocal crosses of *Saccharum* and *Sorghum* in two separate experiments. Gupta et al. (1978) reported the successful production of *Saccharum* x *Sorghum* hybrids in an attempt to breed resistance to shoot fly into tropical cultivated sorghum varieties. Ultimately gene transfer could not be confirmed although is thought to have most likely occurred due to morphological similarities between both parents and the progeny. The first confirmed *Sorghum* x *Saccharum* intergeneric hybrids was reported by Nair (1999). Although hybridization was deemed a success, only five hybrids were recovered from 3,670 pollinated sorghum florets. The hybrid plants lacked vigor and one hybrid seedling died. Ultimately the surviving hybrids were of little use in immediate breeding and had a progeny recovery rate of 0.13% (Nair 1999).

With the rediscovery of *iap* and subsequent development of Tx3361 germplasm, intergeneric hybridization with *Saccharum* has been successful. Over a three year period, Hodnett et al. (2010) produced intergeneric crosses between Tx3361 and sugarcane (*Saccharum* spp) and refined the techniques to eventually produce large and viable quantities of seed. While there had been a few limited reports of intergeneric hybridization of these two crops, this report was unique in that sorghum was used as the female parent and the number of progeny recovered outnumbered any previous reports (thousands vs. < 10). The sugarcane accessions used as male parents consisted of 67 basic and commercial breeding

clones and distinct differences were detected among pollinator parents for their ability to produce viable seed.

Of the putative hybrid seed produced, a total of 1,348 were grown and transplanted in a greenhouse. All plants initially classified as hybrids were later analyzed through somatic chromosome counts which ranged from 56 to 64 and confirmed that these plants were intergeneric hybrids of the two genera. Phenotypically the growth habit resembled that of sugarcane more than sorghum, although most hybrids did possess distinct sorghum characteristics, such as brace roots or smooth leaves.

Objectives

Because of the successful hybridization between sorghum and sugarcane using Tx3361, there is a need to assess the range of functionality of the *iap* locus on other genera in the Poaceae. Since the effect of *iap* is specific to pollen tube germination, pollination and assessment of pollen tube growth provides valuable insight into whether intergeneric hybridization is remotely possible. If pollen tube growth is undetected, then there is no possible chance of hybridization; if it does germinate and produce pollen tube growth into sorghum pistils, then there is a chance of intergeneric hybridization that would merit further study. In addition, Hodnett et al. (2010) documented the differential effect of pollinator genotype on hybridization and the production of progeny. It is likely that these effects will be observed in other genera as well. Given these factors, the objectives of this study are: (i) to screen accessions from different genera within Poaceae to determine the potential for alien pollen tube growth of these genera in Tx3361; and (ii) compare foreign pollen tube growth in pistils of Tx3361 and ATx623 to determine the relative effectiveness of *iap* in allowing the foreign pollen to germinate and grow in sorghum stigmas.

MATERIALS AND METHODS

Plant Material

Sixteen accessions of various species in the genera *Pennisetum, Sorghastrum, Miscanthus*, and *Zea* were selected to use as pollen parents. Pollen parents were selected based on their perceived phylogenetic relationships to sorghum. This germplasm was obtained from the USDA-ARS Plant Germplasm System, various plant breeding programs in the Texas A&M University System and the USDA-ARS Forage Breeding Program at College Station. Plants were maintained in a greenhouse and grown according to practices standard for each species to seasonal maturity. These species were grown separately from the sorghum germplasm before pollination. Flowering of photoperiod insensitive plants (*Z. mays*, *Pennisetum*, *Sorghastrum*) occurred between mid August through early November and flowering of photoperiod sensitive species (*Z. mexicana, Miscanthus*) occurred between late November and early February.

The sorghum genotypes used as female parents were Tx3361*ms3* (Rooney and Kuhlman 2010) and ATx623. Sorghum breeding line ATx623 is an elite inbred female developed and released by Dr. F. R. Miller by the Texas A&M University sorghum breeding program in 1977. Both of these sorghum lines initiate anthesis between 65-75 days after planting. Due to variation in male parent life cycles as well as photoperiod sensitivity of some species, female parents were planted at a variable rate of roughly 15 plants per week to ensure synchronous flowering between males and females. Because ATx623 is cytoplasmic male sterile, each panicle was bagged prior to the onset of anthesis and remained covered until pollination. Tx3361 utilized genetic male sterility and segregated for male and female fertility. At the onset of anthesis each panicle was evaluated for fertility. Those found to be

male fertile were removed from the pots; if male sterile, any portion of the panicle that had flowered was removed and the remainder of the panicle was bagged for pollination. Pollination procedure followed that used by Kuhlman et al. (2010); sterile Tx3361 and ATx623 florets were dusted with freshly collected pollen from male donors no more than two days after anthesis. Florets were isolated on the panicle to ensure adequate pollination by removing florets around those designated for pollination. Approximately 75-100 florets were pollinated in each pollination event.

Pollen Tube Observation Method

Pollinated florets were harvested 24 hours after pollination and immediately placed in a 3:1 (95% ethanol: glacial acetic acid) fixative for a minimum of one week. Pistils were extracted from florets using a dissecting microscope and pistils were processed following a modified version of the protocol described by Kho and Baer (1968) and used by Hodnett et al. (2005) and Kuhlman et al. (2010). Once processed, the pistils were softened overnight in 0.8*M* NaOH. After softening, the pistils were stained in a 0.025% (w/v) aniline blue and 0.1M K_2PO_4 solution for 30 minutes in the dark. Pistils were mounted on microscope slides using a 50% 0.1*M* K_2PO_4 buffer and 50% glycerol mounting medium under 24 x 50 mm coverslips. Pistils on prepared slides were observed using a Zeiss Universal II microscope (Carl Zeiss Inc., Gottingen, Germany) using 10X, 25X and 40X Neofluor objectives.

Fluorescence was induced by a mercury burning lamp emitting 390 to 420- nm light waves and a 450-nm emission filter. The following information was determined: (1) total number of pollen grains on the stigma surface; (2) number of pollen grains that germinated; and (3) the location in the pistil where the most advanced pollen tube grew. Locations within

each pistil at which pollen tube growth was measured were: growth into secondary stigma branches, growth into the stigma axis, growth into the style, and growth into the ovary. During observation, pollen grains and pollen tubes at some data points being taken were too numerous to be counted accurately. Maximum reliable count thresholds were set for data points to ensure accurate measurements. Maximum reliable counts utilized were; 150 pollen grains per pistil, 150 germinated pollen grains per pistil, 100 pollen tubes in secondary stigma branches, 10 pollen tubes in stigma axis, and 10 pollen tubes in styles. No maximum count was set for pollen tube growth into the ovary. Data points for individual pistils exceeding the reliable count thresholds were designated as TNC (Too Numerous to Count) and the maximum reliable count was recorded. Images of pollen germination and pollen tube growth were captured using a MicroFire digital microscope camera equipped with Pictureframe digital imaging software (Optronics, Goleta, California).

Statistical Analysis

Data was analyzed by comparing the number of pollen grains present, the percentage of pollen germination, and pollen tube growth through the pistil. Accessions that did not have any pollen tube growth into the ovary when Tx3361 was the female parent (with the exception of *Miscanthus* species), were not analyzed using ATx623 as the female parent. Accessions with pollen tube growth into the ovary of Tx3361 were subsequently used as pollen donors on ATx623. The use of ATx623 as a female parent served as a numerical as well as visual comparison as to the effectiveness of the *iap* allele on foreign pollen tube growth. These observations were then compared within accession as well as across species within a genus. Statistical analysis of the data was performed using JMP 8 statistical analysis

software in which individual pistils were treated as replicates. Means and standard errors of the percent germination and pollen tube growth throughout the pistil were calculated as well as significant differences between species within each genus and between females within each genotype. Significant differences were calculated across species within a genus in which the same female parent was used as well as for male genotypes in which both female parents were pollinated.. Pistils without any pollen grains were not used for statistical analysis.

RESULTS AND DISCUSSION

Zea mays

A total of six *Zea* accessions were tested as pollen donors on Tx3361. Three were commercially released sweet corn varieties ('Kandy Korn', 'Silver Queen', and 'Tender Treat'), one was an inbred breeding accession (Tx732) and two were from *Z. mexicana* (wild teosinte lines). Although all accession had high levels of pollen grain adhesion and germination, variations in pollen tube growth were noted across accessions. Notably, several accessions had pollen tube growth sufficient in length to complete fertilization although growth into the micropyle was not observed with this method of microscopy and fertilization cannot be confirmed. An initial decrease in the number of pollen tubes was noticed upon entrance into the stigma axis and may be attributed to the overall reduction in the extra cellular matrix (ECM) capacity as well as count limitations due to reliability thresholds placed upon the study.

The variety 'Kandy Korn' had the highest number of pollen tubes growing into the ovary with 1.92% of the pollen tubes reaching the ovary with pollen tubes reaching the ovary in 10 of the 26 pistils examined. Inbred corn line Tx732 had the second highest number of pollen tubes reaching the ovary at 0.76% and pollen tubes reaching the ovary in 6 of the 17 pistils examined. Both *Zea mexicana* accessions had less than 0.1% of the pollen grains reaching the ovary. *Zea mexicana* (PI 566673) had pollen tubes reaching the ovary in 2 of the 19 pistils examined while *Z. mexicana* (PI 566677) had pollen tubes reach the ovary in only 1 out of the 24 pistils examined. Pollen tubes of the varieties 'Silver Queen' and 'Tender Treat' did not grow to the ovary. In fact, pollen tubes of 'Silver Queen' did not

grow past the stigma axis and no 'Tender Treat' pollen tubes were observed past the secondary stigma branches.

When pollen of the *Zea* accessions 'Kandy Korn', Tx732, *Z. mexicana* (PI 566677), and *Z. mexicana* (PI 566673) was placed on the stigmas of ATx623 none of the pollen tubes grew anywhere close to the ovaries. Only 5% of the Tx732 pollen tubes grew into the stigma while *Z. mexicana* (PI 566677) and *Z. mexicana* (PI 566673) had no pollen tube growth into the secondary stigma branches and pollen grain germination rates were 7.7% and 0.43%, respectively. 'Kandy Korn' had five pollen grains observed and none of them germinated. Comparisons between female parents Tx3361 and ATx623 indicate significant differences in pollen germination and tube growth into the stigma branches for all genotypes tested. Statistical differences past the stigma branches were noticed but varied between male genotypes.

Pennisetum

Four *Pennisetum* accessions of two species *P. ciliare* (buffelgrass) and *P. glaucum* (pearl millet) were used as pollen donors on Tx3361. Although all accessions had high levels of pollen grain adhesion and germination, variations in tube growth were noted across all accessions. Both *P. ciliare* accessions had pollen tube growth sufficient in length to enter the embryo sac however, growth into the micropyle was not observed with this method of microscopy and thus fertilization could not be confirmed. An initial decrease in the number of pollen tubes was noticed upon entrance into the stigma axis and this could be attributed to the overall reduction in the extra cellular matrix (ECM) capacity as well as count limitations due to reliability thresholds placed upon the study.

'Frio' buffelgrass had the highest percentage of pollen tubes reaching the ovary at 6.04% of the initial pollen grains growing into the ovary with pollen tubes reaching the ovary in 8 of the 52 pistils observed. The only other accession which had pollen tube growth into the ovary was 'Common' buffelgrass at 3% in which 11 of the 14 pistils observed had pollen tube growth into the ovary. The pearl millet accessions PI 286837 and PI 164410 had no pollen tubes growing to the ovary; however, 1.37% and 0.07% of the pollen tubes reached the stigma axis and style, respectively.

'Common' and 'Frio' buffelgrasses were the only *Pennisetum* species crossed onto ATx623. Neither cultivar had any tube growth into the sorghum pistils and germination rates varied drastically between the two. 'Common' buffelgrass had the highest germination rate of 41.67% and 'Frio' buffelgrass had a germination rate of 6.73%. Comparison of Tx3361 and ATx623 indicate significant differences in tube inhibition at every level of observation when 'Common' buffelgrass is the male parent. Female parents when pollinated with 'Frio' buffelgrass pollen indicate significant differences in pollen germination as well as tube inhibition at the stigma branches and stigma axis but were not significantly different in the style or ovary.

Sorghastrum

Only two *Sorghastrum nutans* (indian grass) accessions were tested as pollen donors on Tx3361. Although all accession had high levels of pollen grain adhesion and germination, variations in pollen tube growth were noted between both accessions. Pollen tube elongation in both accessions appeared to be sufficient in length to complete fertilization although growth into the micropyle was not observed using this method of microscopy and

fertilization could not be confirmed. An initial decrease in the number of pollen tubes was noticed at the stigma axis and this may be attributed to the overall reduction in the extra cellular matrix (ECM) capacity. Pollen tube counts were not high enough to warrant application of reliability thresholds placed on the study.

Of the two *S. nutans* accessions crossed onto ATx3361 neither had pollen tube growth into the ovary. Both accessions did have pollen tube growth into the style but at varying rates. *Sorghastrum nutans* accession 476999 had the highest rate of pollen tube growth into the style at 0.52%. *Sorghastrum nutans* accession 476279 had a pollen tube growth rate into the style of only 0.1%. Due to the low compatibility of the *S. nutans* species tested with Tx3361 no crosses were made onto ATx623 as a comparison in compatibility because it was not deemed necessary for this species.

Miscanthus

A total of four *Miscanthus* accessions, three *M. sinensis* (Chinese plumegrass) and one *M. floridulus* (giant Miscanthus), were tested as pollen donors on Tx3361. Although all accession had high levels of pollen grain adhesion and germination, variations in pollen tube growth were noted across all accessions. An initial decrease in the number of pollen tubes was noticed upon entrance into the stigma axis and this may be attributed to the overall reduction in the extra cellular matrix (ECM) capacity as well as count limitations due to reliability thresholds placed upon the study.

Although all four accessions did have pollen tube growth into the style, none of the four *Miscanthus* accessions tested had pollen tube growth into the ovary. The highest level of pollen tube growth into the style was observed in *M. sinensis* CANE 9233 in which

14.49% of pollen grains produced tubes that grew to the style. *Miscanthus sinensis* (PI 294602), *M. sinensis* (PI 295764), and *M. floridulus* (PI 230189) had pollen tube growth rates into the style of 9.47%, 6.14%, and 4.88%, respectively.

Even though none of the accessions had tube growth into the ovary, [*M. floridulus* (PI 230189), *M. sinensis* (PI 295764), and *M. sinensis* (CANE 9233)] were crossed onto ATx623. Only two accessions [*M. sinensis* (CANE 9233) and *M. sinensis* (PI 295764)] had pollen tubes entering the pistil and neither had pollen tubes growing past the secondary stigma branches. *Miscanthus sinensis* (CANE 9233) had a pollen tube growth rate into the secondary stigma branches of 1.83% and *M. sinensis* (PI 295764) had 0.25% of its pollen tubes growing to the secondary stigma branches. Comparison of Tx3361 and ATx623 indicated significant differences in pollen germination and tube inhibition at every level of observation except the ovary for all three *Miscanthus* accessions tested.

CONCLUSIONS

The rate and location of pollen tube inhibition varies not only between species but also within species. Location of pollen tube inhibition appears to be of lesser importance than rate of inhibition within most accessions. As a result of this study, it was determined that compatibility cannot be applied across entire genera. Variation in pollen tube growth between genera was expected and was noticed. Many factors may contribute to this variation including, but not limited to, the pre-zygotic factors mentioned by Hodnett et al. (2005). Pre-zygotic factors between the genera will differ based on the primary method of pollination for the genera in question and may vary further if multiple methods of pollination are utilized within a single genus. These morphological characteristics define and separate the genera from each other and are utilized to maintain that separation. Further incompatibilities between the species such as pollen tube length in comparison to distance to the ovary may be further sources of variation between the genera as noted by Dhaliwal and King (1978). Lastly, it should be noted that differences in life cycles as well as photoperiod sensitivity of the plants may be contributing factors to incompatibility as this determined the date of pollination in some species tested.

Successes and failures occurred within some genera as well as successes and failures within species with success being regarded as pollen tube growth into the ovary. The reproductive barriers observed do not remain consistent throughout each genus or within species. This result indicates that assumptions may not be applied across a species but must be done on an individual genotype basis and that variation still exists within closely related species. Species that were tested within each genera do not differ drastically in their morphological characteristics (with the exception of *Z. Mexicana*) but do contain genetic

variation which ensures variation across the species. The noticeable variation of pollen-pistil compatibility within a species can most likely be attributed to this genetic variation between accessions due to natural and intentional selection of the individual accession. All of the factors listed above contribute to the increased or decreased intensity of the pre-zygotic factors influencing pollen-pistil compatibility.

Data from this study indicates that in comparison to ATx623, presence of the *iap* allele in Tx3361 greatly reduces the reproductive barriers within *S. bicolor*. Results indicate that in the absence of the *iap* allele pollen tube growth is not only reduced but in some cases completely prevented. The presence of the *iap* allele appears to have the most dramatic effect on pollen germination with some species germinating at or near 100% and considerable pollen tube growth in the secondary stigma branches. When *iap* is absent pollen germination is greatly reduced and even prevented completely in some species. In species that did have pollen grains germinate without the *iap* present, only three species produced pollen tubes that grew into the secondary stigma branches and only one had pollen tubes grow into the stigma axis. The use of backcrossing to introgress the *iap* allele into ATx623 ensures that these two genotypes are of the same lineage. Genetic variation between these two lines has been minimized as a result of backcross breeding to derive Tx3361 in which ATx623 was the recurrent parent. Due to this method of development, an increase in foreign pollen tube compatibility is a direct result of the *iap* allele.

The ability to fertilize *S. bicolor* with alien species could not be determined in this study. The final barrier in reproduction is the growth of the pollen tube into the embryo sac and subsequent fertilization which occurs in the female gametophytes and cannot be observed with microscopic methods used in this study. While it was observed and served as

a basis for determining the effectiveness of the *iap* allele, it is not appropriate to state that high levels of pollen tubes within the ovary increase the chance of fertilization due to the inability to observe this final barrier. Further studies are required to determine the overall ability of the *iap* in allowing fertilization of *S. bicolor* by foreign species. Due to reproductive barrier inconsistencies within each species, it is most appropriate to test more accessions within a species rather than increasing the number of species within a genus in future studies. Furthermore, once it is observed that pollen tubes do reach the ovary of a cross, observation should be continued to determine if fertilization has occurred. Fertilization may be determined using paraffin sectioning as was demonstrated by Burson (1987). Should fertilization occur with the use of the *iap* allele, it may be necessary to perform embryo rescue to recover successful hybrid progeny as in previous studies.

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APPENDIX

Table 1. Genus and species identification of plant material used as male and female parents.

Genus	Common Name	PI # / accession	Reference Code	Source Location
Pennisetum				
P. ciliare	Buffelgrass	Frio	Buffel Frio	USDA-ARS Forage Breeding Program
P. ciliare	Buffelgrass	Common	Buffel Common	USDA-ARS Forage Breeding Program
P. glaucum	Pearl millet	PI 286837	P. glaucum-1	National Plant Germplasm Program
P. glaucum	Pearl millet	PI 164410	P. glaucum--2	National Plant Germplasm Program
Sorghastrum				
S. nutans	Indiangrass	PI 476279	Indian 476279	USDA-ARS Forage Breeding Program
S. nutans	Indiangrass	PI 476999	Indian 47699	USDA-ARS Forage Breeding Program
Miscanthus				
M. floridus	Giant Miscanthus	PI 230189	M. floridulus-1	National Plant Germplasm Program
M. sinensis	Chinese plumegrass	PI 295764	M. sinensis-1	National Plant Germplasm Program
M. sinensis	Chinese plumegrass	CANE 9233	M. sinensis-2	National Plant Germplasm Program
M. sinensis	Chinese plumegrass	PI 294602	M. sinensis-4	National Plant Germplasm Program
Zea				
Z. mays	Sweet corn	Kandy Korn	Kandy Korn	Commercial Hybrid Release
Z. mays	Sweet corn	Silver Queen	Silver Queen	Commercial Hybrid Release
Z. mays	Sweet corn	Tender Treat	Tender Treat	Commercial Hybrid Release
Z. mays	Corn	Tx-732	Tx732 mays	TAMU Corn Breeding Program
Z. mexicana	Teosinte	PI 566677	Z. mexicana-3	National Plant Germplasm Program
Z. mexicana	Teosinte	PI 566673	Z. mexicana-1	National Plant Germplasm Program
Sorghum				
S. bicolor	Sorghum	Tx3361	Tx3361	TAMU Sorghum Breeding Program
S. bicolor	Sorghum	ATx623	ATx623	TAMU Sorghum Breeding Program

† Reference codes indicate designation of species during the experiment used to cross reference Plant Identification (PI) numbers.

‡ TAMU refers to Texas A&M University at College Station, Texas.

Table 2. Means and standard deviation of pollen germination and pollen tube growth of *S. bicolor* (*iap,iap*) and *S. bicolor* (*Iap*,__) x *Zea* species.

Cross	Pistils Observed	Pollen Grains Observed	Pollen Germination	Pollen tube growth to			
				Stigma Branches	Stigma Axis	Style	Ovary
	no.		%				
S. bicolor (*iap*,*iap*)							
Tx3361 x *S. bicolor*	12	487	97.81 ± 3.75a	83.49 ± 5.91a	32.22b ± 21.34a	20.22 ± 17.81a	6.71 ± 13.93a
Tx3361 x Kandy Korn	26	>1572	92.93 ± 4.06 ab	52.6 ± 5.09c	9.21 ± 2.35cd	3.42 ± 1.33 b	1.92 ± 1.27b
Tx3361 x Silver Queen	9	586	90.78 ± 1.88ab	83.34 ± 2.15a	17.71 ± 2.31b	0.00 ± 0.00 b	0.00 ± 0.00b
Tx3361 x Tender Treat	12	314	56.99 ± 3.76c	17.32 ± 2.39d	0.00 ± 0.00e	0.00 ± 0.00 b	0.00 ± 0.00b
Tx3361 x Tx732 mays	17	>1404	87.93 ± 3.48 b	69.92 ± 5.83b	12.68 ± 2.00bc	2.97 ± 0.51 b	0.76 ± 0.28b
Tx3361 x *Z. mexicana*-1	19	>2850	†100 ± 0.00a	†66.67 ± 0.00bc	†6.67 ± 0.00bc	1.54 ± 0.8 b	0.07 ± 0.21b
Tx3361 x *Z. mexicana*-3	24	>2900	97.49 ± 0.80ab	60.01 ± 1.95c	1.25 ± 0.31de	0.02 ± 0.02 b	0.02 ± 0.02b
S. bicolor (IAP,___)							
ATx623 x *S. bicolor*	7	260	96.43 ± 3.57a	88.38 ± 7.53a	30.13 ± 9.91a	18.14 ± 4.18a	6.45± 5.99a
ATx623 x Kandy Korn	4	5	0.00 ± 0.00c	0.00 ± 0.00bc	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b
ATx623 x Tx732 mays	10	15	45.00 ± 15.72b	15.00 ± 10.67b	5.00 ± 5.00b	0.00 ± 0.00b	0.00 ± 0.00b
ATx623 x *Z. mexicana*-1	15	27	7.70 ± 20.70c	0.00 ± 0.00c	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b
ATx623 x *Z. mexicana*-3	23	77	0.43 ± 0.43c	0.00 ± 0.00c	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b

† (*) indicates maximum reliable pollen tube count was reached for all observations.

‡ (>) indicates maximum reliable pollen grain count was reached for one or more observations.

§ Letters within columns indicate significant difference between the groups based on LSD, $P<.05$.

Table 3. Means and standard deviation of pollen germination and pollen tube growth of *S. bicolor* (*iap*,*iap*) and *S. bicolor* (*Iap*,__) x *Zea* species.

Cross	Pistils Observed	Pollen Grains Observed	Pollen Germination	Pollen tube growth to			
				Stigma Branches	Stigma Axis	Style	Ovary
	no.		%				
Kandy Korn							
Tx3361 x Kandy Korn	26	>1572	92.93 ± 4.06 a	52.6 ± 5.09a	9.21 ± 2.35a	3.42 ± 1.33a	1.92 ± 1.27a
ATx623 x Kandy Korn	4	5	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Tx732 mays							
Tx3361 x Tx732 mays	17	>1404	87.93 ± 3.48a	69.92 ± 5.83a	12.68 ± 2.00a	2.97 ± 0.51 a	0.76 ± 0.28a
ATx623 x Tx732 mays	10	15	45.00 ± 15.72b	15.00 ± 10.67b	5.00 ± 5.00a	0.00 ± 0.00b	0.00 ± 0.00a
Z. mexicana-1							
Tx3361 x *Z. mexicana*-1	19	>2850	†100 ± 0.00a	†66.67 ± 0.00a	†6.67 ± 0.00a	1.54 ± 0.8a	0.07 ± 0.21a
ATx623 x *Z. mexicana*-1	15	27	7.70 ± 20.70b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00a
Z. mexicana-3							
Tx3361 x *Z. mexicana*-3	24	>2900	97.49 ± 0.80a	60.01 ± 1.95a	1.25 ± 0.31a	0.02 ± 0.02a	0.02 ± 0.02a
ATx623 x *Z. mexicana*-3	23	77	0.43 ± 0.43b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00a	0.00 ± 0.00a

† (*) indicates maximum reliable pollen tube count was reached for all observations.

‡ (>) indicates maximum reliable pollen grain count was reached for one or more observations.

§ Letters within columns indicate significant difference between the groups based on LSD, $P<.05$.

Table 4. Means and standard deviation of pollen germination and pollen tube growth of *S. bicolor* (*iap,iap*) and *S. bicolor* (*Iap*,__) x *Pennisetum* species.

Cross	Pistils Observed	Pollen Grains Observed	Pollen Germination	Pollen tube growth to			
				Stigma Branches	Stigma Axis	Style	Ovary
	no.		%				
S. bicolor (*iap,iap*)							
Tx3361 x *S. bicolor*	12	487	97.81 ± 3.75a	83.49 ± 5.91a	32.22 ± 21.34a	20.22 ± 17.81a	6.71 ± 13.93a
Tx3361 x Buffel Common	14	>1800	†100 ± 0.00ab	63.67 ± 8.10ab	†6.67 ± 0.00abc	3.95 ± 3.23ab	3.00 ± 2.78a
Tx3361 x Buffel Frio	52	346	81.58 ± 24.30b	37.14 ± 35.84bc	20.30 ± 29.82b	8.45 ± 18.78b	6.04 ± 18.78a
Tx3361 x *P.glaucum*-1	24	790	79.43 ± 14.01b	18.40 ± 14.20d	1.37 ± 5.17c	0.00 ± 0.00b	0.00 ± 0.00a
Tx3361 x *P.glaucum*-2	22	1661	81.94 ± 12.69b	31.20 ± 12.20cd	0.24 ± 0.85bc	0.07 ± 0.35b	0.00 ± 0.00a
S. bicolor (IAP,___)							
ATx623 x *S. bicolor*	7	260	96.43 ± 3.57a	88.38 ± 7.53a	30.13 ± 9.91a	18.14 ± 4.18a	6.45± 5.99a
ATx623 x Buffel Common	8	19	41.67 ± 11.78b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b
ATx623 x Buffel Frio	17	74	6.73 ± 14.50b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b

† (*) indicates maximum reliable pollen tube count was reached for all observations

‡ (>) indicates maximum reliable pollen grain count was reached for one or more observations

§ Letters within columns indicate significant difference between the groups based on LSD, $P<.05$.

Table 5. Means and standard deviation of pollen germination and pollen tube growth of *S. bicolor* (*iap,iap*) and *S. bicolor* (*Iap*,__) x *Pennisetum* species.

Cross	Pistils Observed	Pollen Grains Observed	Pollen Germination	Pollen tube growth to			
				Stigma Branches	Stigma Axis	Style	Ovary
	no.		%				
Buffel Common							
Tx3361 x Buffel Common	14	>1800	†100 ± 0.00a	63.67 ± 8.10a	†6.67 ± 0.00a	3.95 ± 3.23a	3.00 ± 2.78a
ATx623 x Buffel Common	8	19	41.67 ± 11.78b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b
Buffel Frio							
Tx3361 x Buffel Frio	52	346	81.58 ± 24.30a	37.14 ± 35.84a	20.30 ± 29.82a	8.45 ± 18.78a	6.04 ± 18.78a
ATx623 x Buffel Frio	17	74	6.73 ± 14.50b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00a	0.00 ± 0.00a

† (*) indicates maximum reliable pollen tube count was reached for all observations

‡ (>) indicates maximum reliable pollen grain count was reached for one or more observations

§ Letters within columns indicate significant difference between the groups based on LSD, $P<.05$.

Table 6. Means and standard deviation of pollen germination and pollen tube growth of *S. bicolor* (*iap,iap*) and *S. bicolor* (*Iap*,__) x *Sorghastrum* species.

Cross	Pistils Observed	Pollen Grains Observed	Pollen Germination	Pollen tube growth to			
				Stigma Branches	Stigma Axis	Style	Ovary
	no.		%				
S. bicolor (*iap,iap*)							
Tx3361 x *S. bicolor*	12	487	97.81 ± 3.75 a	83.49 ± 5.91 a	32.22b ± 21.34 a	20.22 ± 17.81 a	6.71 ± 13.93 a
Tx3361 x Indian476279	20	>2202	52.96 ± 19.54 b	29.70 ± 12.68 b	9.14 ± 6.01 b	0.10 ± .32 b	0.00 ± 0.00 b
Tx3361 x Indian476999	47	>2388	31.47 ± 20.39 c	7.50 ± 10.23 c	1.31 ± 3.23 c	0.52 ± 1.50 b	0.00 ± 0.00 b

† (>) indicates maximum reliable pollen grain count was reached for one or more observations.

‡ Letters within columns indicate significant difference between the groups based on LSD, $P<.05$

Table 7. Means and standard deviation of pollen germination and pollen tube growth of *S. bicolor* (*iap,iap*) and *S. bicolor* (*Iap*,__) x *Miscanthus* species.

Cross	Pistils Observed	Pollen Grains Observed	Pollen Germination	Pollen tube growth to			
				Stigma Branches	Stigma Axis	Style	Ovary
	no.		%				
S. bicolor (*iap,iap*)							
Tx3361 x *S. bicolor*	12	487	97.81 ± 3.75a	83.49 ± 5.91a	32.22b ± 21.34b	20.22 ± 17.81a	6.71 ± 13.93a
Tx3361 x *M.floridulus*-1	13	1366	84.56 ± 4.62c	67.79 ± 5.55b	37.83 ± 9.48a	4.88 ± 3.11b	0.00 ± 0.00ab
Tx3361 x *M.sinensis*-1	11	>1570	99.09 ± 3.01ab	67.35 ± 2.29bc	7.35 ± 2.29b	6.14 ± 0.92ab	0.00 ± 0.00ab
Tx3361 x *M.sinensis*-2	22	645	81.00 ± 9.68c	46.16 ± 9.89d	23.71 ± 8.03c	14.49 ± 9.54b	0.00 ± 0.00b
Tx3361 x *M.sinensis*-4	21	>777	85.91 ± 10.56bc	54.39 ± 12.31c	29.24 ± 13.18b	9.47 ± 7.20b	0.00 ± 0.00b
S. bicolor (IAP,___)							
ATx623 x *S. bicolor*	7	260	96.43 ± 3.57a	88.38 ± 7.53a	30.13 ± 9.91a	18.14 ± 4.18a	6.45± 5.99a
ATx623 x *M.floridulus*-1	6	10	16.67 ± 25.81c	0.00 ± 0.00b	0.00 ± 0.00c	0.00 ± 0.00b	0.00 ± 0.00b
ATx623 x *M.sinensis*-1	22	128	31.59 ± 24.04c	0.25 ± 0.25b	0.00 ± 0.00c	0.00 ± 0.00b	0.00 ± 0.00b
ATx623 x *M.sinensis*-2	21	285	51.33 ± 20.47b	1.83 ± 3.27b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b

† (>) indicates maximum reliable pollen grain count was reached for one or more observations.

‡Letters within columns indicate significant difference between the groups based on LSD, $P<.05$.

Table 8. Means and standard deviation of pollen germination and pollen tube growth of *S. bicolor* (*iap*,*iap*) and *S. bicolor* (*Iap*,__) x *Miscanthus* species.

Cross	Pistils Observed	Pollen Grains Observed	Pollen Germination	Pollen tube growth to			
				Stigma Branches	Stigma Axis	Style	Ovary
	no.		%				
M. floridulus-1							
Tx3361 x *M.floridulus*-1	13	1366	84.56 ± 4.62a	67.79 ± 5.55a	37.83 ± 9.48a	4.88 ± 3.11a	0.00 ± 0.00a
ATx623 x *M.floridulus*-1	6	10	16.67 ± 25.81b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00a
M. sinensis-1							
Tx3361 x *M.sinensis*-1	11	>1570	99.09 ± 3.01a	67.35 ± 2.29a	7.35 ± 2.29a	6.14 ± 0.92a	0.00 ± 0.00a
ATx623 x *M.sinensis*-1	22	128	31.59 ± 24.04b	0.25 ± 0.25b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00a
M. sinensis-2							
Tx3361 x *M.sinensis*-2	22	645	81.00 ± 9.68a	46.16 ± 9.89a	23.71 ± 8.03a	14.49 ± 9.54a	0.00 ± 0.00a
ATx623 x *M.sinensis*-2	21	285	51.33 ± 20.47b	1.83 ± 3.27b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00a

† (>) indicates maximum reliable pollen grain count was reached for one or more observations.

‡Letters within columns indicate significant difference between the groups based on LSD, $P<.05$.

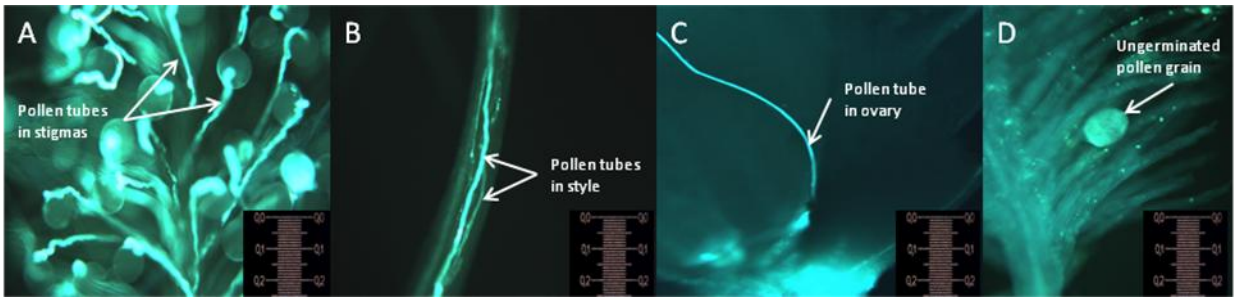

Fig. 1. Pollen tube growth of intergeneric crosses of *S. bicolor* x *Zea mays* var. 'Kandy Korn'. (A) Pollen grains that germinated and pollen tubes that have grown into secondary stigma branches of Tx3361. (B) Pollen tubes of that grew down the style of Tx3361. (C) Pollen tubes of that have grown into and toward the base of the ovary of TX3361. (D) An ungerminated pollen grain on the secondary stigma branch of ATx623. Micrometer readings are in millimeters.

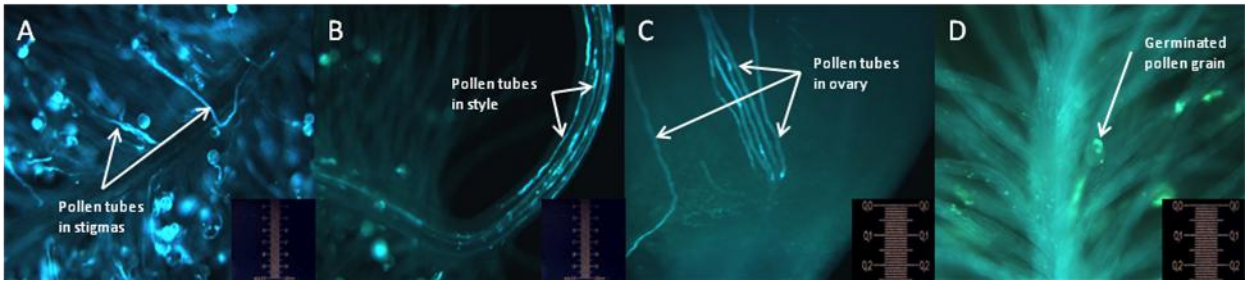

Fig. 2. Pollen tube growth of intergeneric crosses of *S. bicolor* x *Pennisetum ciliare* accession 'Common buffelgrass'. (A) Germinated pollen grains and pollen tubes that grew into the secondary stigma branches and down the stigma axis of Tx3361. (B) Pollen tubes that have grown into the style of Tx3361. (C) Pollen tubes that have grown into the ovary of Tx3361. (D) A germinated pollen grain on the secondary stigma branch of ATx623.

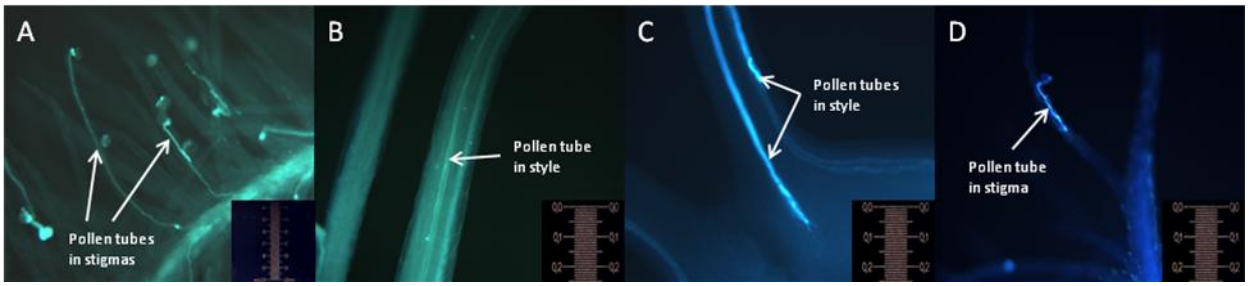

Fig. 3. Pollen tube growth of intergeneric crosses in *S. bicolor* x *Miscanthus floridulus* (PI 230189). (A) Germinated pollen grains and pollen tubes that grew into the secondary stigma branches and down the stigma axis of Tx3361. (B) Pollen tubes that have grown into the style of Tx3361. (C) Pollen tubes that have reached the base of the style but did not enter the ovary of Tx3361. (D) Germinated pollen grain and pollen tube that has grown into the secondary stigma branch of ATx623. Micrometer readings are in millimeters.

VITA

Matthew Scott Bartek received his Associate of Applied Science degree in golf course and landscape management from Texas State Technical College at Waco Texas in 2002. He entered the Department of Soil and Crop Science at Texas A&M University in 2003 and received his Bachelor of Science degree in agronomy in 2008 and his Master of Science degree in sorghum breeding and genetics in 2010. His research interests include sorghum breeding with emphasis on cytology and field based breeding. Matthew will continue his graduate education in the doctoral program for a Doctor of Philosophy degree in sorghum breeding and genetics at Texas A&M University.

Mr. Bartek may be reached at: Texas A&M University Department of Soil and Crop Sciences 370 Olsen Blvd. 2474 TAMU College Station, TX 77843-2474. His email address is mbartek@neo.tamu.edu.